World Journal of Microbiology and Biotechnology (2021) 37:123
https://doi.org/10.1007/s11274-021-03094-3

ORIGINAL PAPER

Residual biomass from surfactin production is a source of arginase and adsorbed surfactin that is useful for environmental remediation

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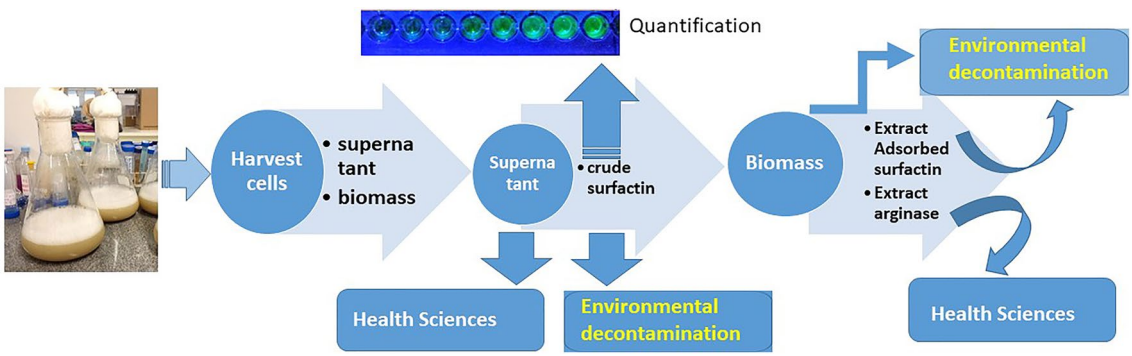
Received: 15 January 2020 / Accepted: 1 June 2021 / Published online: 23 June 2021

Abstract

Lipopeptides are important secondary metabolites produced by microbes. They find applications in environmental decontamination and in the chemical, pharmaceutical and food industries. However, their production is expensive. In the present work we propose three strategies to lower the production costs of surfactin. First, the coproduction of surfactin and arginase in a single growth. Second, extract the fraction of surfactin that adsorbs to the biomass and is removed from the growth medium through centrifugation. Third, use microbial biomass for the remediation of organic and inorganic contaminants. The coproduction of surfactin and arginase was evaluated by factorial design experiments using the LB medium supplemented with arginine. The best conditions for surfactin production were 22 h of growth at 37 °C using LB supplemented with arginine 7.3 g/L. Almost similar conditions were found to produce highest levels of arginase, 24 h and 6.45 g/L arginine. Decontamination of phenol and copper from artificial samples was attained by treatment with residues from lipopeptide production. Thus, cell suspensions and wash-waters used to extract surfactin from the biomass. Cell suspensions were used to successfully remove hydroquinone. Cell suspensions and wash-waters containing surfactin were successfully used to recover copper from solution. Specific monitoring methods were used for phenol and metal solutions, respectively a biosensor based on tyrosinase and either atomic absorption flame ionization spectrometry or absorbance coupled to the Arduino™ platform. Therefore, we report three alternative strategies to lower the production costs in lipopeptide production, which include the effective recovery of copper and phenol from contaminated waters using residues from surfactin production.

Graphic abstract

Sustainable and profitable production of surfactin can be achieved by a coproduction strategy of lipopeptides and enzymes. Lipopeptides are collected in the supernatant and enzymes in the biomass. In addition, lipopeptides that coprecipitate with biomass can be recovered by washing. Lipopeptide wash-waters find applications in remediation and cells can also be used for environmental decontamination.



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Keywords Lipopeptides · Surfactin · Arginase · Microbial biomass · Copper · Hydroquinone · Environmental decontamination

Introduction

World's global challenges are infectious diseases, cancer, food production and environmental decontamination (Waldvogel 2004; Bagatin et al. 2014; Uyttendaele et al. 2015). Biotechnology is a growing industry that can help in many ways to tackle all these challenges. The search and production of novel antibiotics, anti-cancer drugs, food production and environmental decontamination processes all benefit from biotechnological processes (Silber et al. 2016).

Microbial secondary metabolites are important molecules produced by biotechnology. Some of these molecules, like the lipopeptides produced by Bacillus spp., have important biological properties as antimicrobial, antitumoral and antiviral activities. For instance, surfactins have been described as the most potent biosurfactants produced naturally. These lipopeptides are produced non-ribosomally by multifunctional peptide synthetases (Koumoutsi et al. 2004; Sen 2010). Their commercial production has been improved in the last 5 years by novel biotechnological approaches. Still it represents an expensive process but, considering the growing number of applications of these molecules, it is expected that the world demand will justify an ever-growing production.

To tackle production costs, an aspect that can be explored is the coproduction of more than one biotechnological product. This approach has been elegantly suggested by Qi and Liang (2015) as single cell biorefinery (Qi and Liang 2015). An example of such biorefinery process was the use of *B. subtilis* for the coproduction of polyhydroxyalkanoate and single cell proteins (Umesh et al. 2017).

When the genomes of *B. subtilis* and *B. amyloliquefaciens* (*velezensis*) are analyzed, the presence of several gene clusters involved in secondary metabolite biosynthesis is evident (Pan et al. 2017). The co-expression of these genes gives rise to the best performance of the strains in biological control (Magno-Pérez-Bryan et al. 2015).

Besides the production of secondary metabolites, *Bacillus* spp. are known to produce several hydrolases (including keratinases) (Vidmar and Vodovnik 2018) with applications in food, hygiene/health, energy production, and environmental remediation (Yu et al. 2013; Parthipan et al. 2017; Marathe et al. 2018).

Microbial enzymes have the advantage of being easy to produce. They are also, generally non-toxic and have stability (Rey et al. 2004; Singh et al. 2016). Besides the applications in several industrial processes, microbial enzymes can be used in medicine as in the treatment of digestive disorders, skin ulcers and cancer (Singh et al. 2016).

The use of microbial enzymes to treat cancer has been described and is currently a common protocol for leukemia treatment (Bernardes et al. 2010). The treatment is based on the reduction of the levels of the amino acid asparagine, generating aspartic acid and ammonia. As the cancer cells cannot replace or synthesize asparagine from aspartic acid, they cannot survive (Egler et al. 2016). The enzymes arginase and arginine deiminase have similar applications, however, based on reduction of arginine levels (Stasyk et al. 2015). All three enzymes, asparaginase, arginase and arginine deiminase can be produced by Bacillus spp. (Maghnouj et al. 1998; Yu et al. 2013; Qeshmi et al. 2016).

The aim of this work was to demonstrate that B. *velezensis* 0G can grow and produce concomitantly surfactin and arginase. Surfactin is released in the growth medium, leaving behind, the microbial biomass, which is a source of several enzymes, including arginase. Here, we demonstrate that the separation process is appropriate, as the biosurfactant remains in the culture supernatant and the enzyme stays in the biomass. The coproduction strategy is based on the biosynthesis of other amino acids using arginine as the starting material. Arginase acts on arginine to produce ornithine, which is a precursor of L-glutamate (Ginguay et al. 2017), a substrate for surfactin biosynthesis. Therefore, arginine was added as supplement in the growth medium. This approach was used aiming both at the induction of arginase expression and production of surfactin. We also demonstrate that, besides being a valuable source of enzymes, the cells are also useful for decontamination of phenol and metals. In addition, it was shown that harvesting the cells by centrifugation, is a step that reduces the yield of surfactin, since a fraction of lipopeptide is adsorbed to the biomass and coprecipitates.

Materials and methods

Cells and growth conditions

The strain *Bacillus amyloliquefaciens* 0G, which is currently named *B. velezensis* OG (Etchegaray et al. 2008; Etchegaray et al. 2017) was used as a source of surfactin and arginase. For initial experiments, growth was carried out in Modified Landy's medium, using glycerol 20 g/L and arginine 5 g/L to replace respectively, glucose and glutamic acid. For the design experiments, the Luria–Bertani (LB medium), which has protein hydrolysates (Sezonov et al. 2007) was used as a base medium that was supplemented with the amino acid

L-arginine. Unless otherwise stated, all reagents were purchased from Sigma-Aldrich (Brazil).

Genomic DNA information of B. velezensis strain 0G

The DNA sequence of *B. velezensis* has been deposited on the National Center of Biotechnology information (NCBI). The bio-project's accession number for this genome is PRJNA518012.

Growth conditions, DNA extraction and genome sequencing

For DNA extraction and genome sequencing, *B. velezensis* strain 0G was grown in modified Landy´s medium. Growth was carried out at 37 °C for 72 h, with normal aeration at 220 rpm. DNA was extracted with the AxyPrep Bacterial Genomic DNA Miniprep Kit (Axygen Biosciences) according to manufacturer's instructions. The extracted DNA was used for the construction of a paired-ends library with the Nextera XT DNA Sample Prep Kit (Illumina) and genome sequencing was carried out in the MiSeq platform using the MiSeq Reagent Kit v3 (Illumina) following instructions provided by the manufacturer. The reads obtained were processed and bases with qualities lower than Phred 28 and sequences shorter than 50 bp were eliminated using PRINSEQ 0.20.4 (Schmieder and Edwards 2011).

Genome assembly and annotation

Genome assembly was carried out with SPAdes 3.11.0 (Bankevich et al. 2012) and Platanus 1.2.4 (Kajitani et al. 2014). The assembled genome was automatically annotated with Prokka 1.11 (Seemann 2014) and gene clusters putatively encoding secondary metabolite biosynthesis were predicted with antiSMASH 5.1.2 (Blin et al. 2013, 2017). Manual curation of annotations was performed with BLAST 2.6.0 + (Altschul et al. 1990; Camacho et al. 2009) and Artemis 16.0.0 (Rutherford et al. 2000; Carver et al. 2012), based on alignments against a custom database and the NCBI GenBank (Boratyn et al. 2013; NCBI Resource Coordinators 2017).

Estimating surfactin production using a colorimetric method

To evaluate the production of surfactin by *B. velezensis* strain 0G, the growth was carried out for 24 h using the modified Landy's medium described above. At the end of the growth, cells were collected by centrifugation at 9000×*g* for 10 min at 4 °C. The supernatant was used as a source of soluble surfactin. The biomass was used as additional source of surfactin after a series of washes that involved resuspension in 10 mL of 25 mM Tris.HCl, pH 7.5 (buffer A) and centrifugation at 9000×*g* for 10 min at 4 °C. The soluble fraction of surfactin, and the fraction that was extracted from the biomass were quantified by the colorimetric method using the surfactant-dye complex of Cetylpyridinium chloride-Fluorescein (CPCl-FL) (Heuson et al. 2019) with adaptation. Instead of fluorescence, in this work, we have measured the absorption of liberated fluorescein at 490 nm. The linear equation (y = 0.0006X—0.0066), was used to estimate surfactin production in the supernatant and recovered from biomass.

Extraction and determination of arginase activity

The biomass collected by centrifugation was washed with buffer A and resuspended in the same buffer, containing lysozyme 1 mg/mL, Triton-X-100 0.1%, and glass beads. The suspension was maintained at 4 °C and submitted to a water sonicator (Ciencor, Brazil). A series of sonications and resting steps of 60 s were performed for 10 min. After this step, the broken cells were removed by centrifugation and the soluble extract was treated as a source of arginase for determination of enzymatic activity. Reactions were started by manganese activation for 15 min at 55 °C and contained 25 µL of crude enzyme and 25 µL of manganese 10 mM (pH adjusted to 7.5 in Tris.HCl). This was followed by the addition of arginine 20 mM, pH 8.5, adjusted with Trizma base (Sigma Aldrich, Brazil) and incubation at 37 °C for 30 to 60 min. The enzyme reaction was stopped by the addition of a mixture of sulfuric acid and phosphoric acid 400 µL (Iyamu et al. 2008) and 25 µL of ISPF 9% (w/v) in 100% ethanol. After incubation at 95 °C for 60 min, the reaction was transferred to a dark place and incubated for 10 min at room temperature. Then, 200 µL of each reaction was transferred to microtiter plates and absorbance was determined at 450 nm. Quantification was attained by a calibration curve prepared with urea and treated essentially as the reaction mixtures. One unit of arginase corresponds to the amount of enzyme that produces 1 µmol of urea per min at 37 °C.

Factorial design experiments for the coproduction of surfactin and arginase

The coproduction of surfactin and arginase by *B. velezensis* 0G was evaluated using two different parameters, respectively growth time and supplementation with arginine in LB medium. The study was based on factorial design (star-type) 2^2 with central and axial points. Cultivation

Table 1 Factorial design 2^2 experimental parameters

	Parameters (37 °C)				
Indicators	− 1.42	− 1	0	1	1.42
L-Arginine (g/L)	0.5	2	5	8	9,5
Time (h)	18	20	24	28	30

Table 2 Experimental conditions used in the Factorial design experiments

Assay	L-arginine (X)	Time (Y)
1	− 1	− 1
2	1	− 1
3	− 1	1
4	1	1
5	0	0
6	0	0
7	0	0
8	0	0
9	− 1.42	0
10	0	− 1.42
11	1.42	0
12	0	1.42

of *B. velezensis* was carried out in 250 mL conical flasks containing 25 mL of LB that was supplemented with arginine. Growth was carried out a 37 °C at 250 rpm. Sample collection was carried out between 18 and 30 h of growth. Each time point was collected by removing the flask and harvesting the microbial biomass by centrifugation. The supernatant was the source of surfactin and the pellet, the source of arginase, as the enzyme is segregated within the cells (Yu et al. 2013). Surfactin was quantified by the colorimetric method described above and arginase was extracted from the biomass in Buffer A, containing 0.1% triton X-100 and lysozyme (described above). Arginine supplementation varied from 0.5 to 9.5 g/L. The central point (0.0) for cultivation time was set at 24 h and at an arginine concentration of 5 g/L. Table 1 presents the levels for each parameter. Based on this information, the medium composition and growth time for each sample were determined for the 12 culture flasks. The experimental plan for each sample is shown on Table 2. The central points correspond to flasks 5 to 8.

Applications of residues from surfactin production for environmental decontamination

Decontamination of copper

Microbial cells that were collected and washed with buffer A and that were not used for arginase extraction, were used for environmental decontamination experiments. These were boiled for 10 min and resuspended in buffer A at 10% (w/v) to be used for copper decontamination. In addition, the surfactin wash-waters were also used for copper decontamination.

Decontamination of hydroquinone

As described above, microbial biomass was washed once with buffer A, boiled for 10 min, and centrifuged. The pellet composed of wet cells was weighted and transferred to a volume of 1 mL of buffer A to make respectively a 5% and 30% cell suspension (w/v). These were then added to a sample containing hydroquinone (50 ppm) in the proportion of 1:1, or 500 µL each.

Specific quantification of copper and hydroquinone

Copper quantification

The method of atomic absorption spectrophotometry was used to quantify copper (Hassanpoor and Khayatian 2014). Analytical curves were prepared by dilution of a standard copper solution (1000 ppm). The final concentrations of copper used in the analytical curve varied from 0.6 to 3.5 ppm.

Hydroquinone quantification

For the determination of hydroquinone, before and after treatment, a biosensor was assembled on carbon paste, which contained immobilized tyrosinase, physically adsorbed to iron magnetic nanoparticles (Mendes et al. 2017). Hydroquinone is a strong inhibitor of tyrosinase, and this is the basis for its quantitative detection (Zolghadri et al. 2019). A calibration curve was prepared using standard solutions of hydroquinone.

Decontamination of copper monitored by Arduino™

The Arduino UNO, which has 54 pins for connection and 16 analogic connections, was used with an Ethernet shield W5100. The system was assembled using two Arduino platforms, each one to control one LED lamp introduced

in the detection chamber. The detection chamber is shown in Fig. 1. It was built on a wood box with a lid, to avoid external light. Two blue LEDs with maximal absorption of 490 nm were attached, respectively to the top and the bottom of the wood chamber. In this configuration, it is possible to measure simultaneously the absorbance at the top and at the bottom of the chamber. A difference between these two measurements indicates precipitation of the suspension.

Results

Biosurfactant production

The genome analysis of *B. velezensis* strain 0G using the platform antiSMASH 5.1.2 indicates that this strain has gene clusters associated with the production of several antimicrobial peptides and other secondary metabolites. Among these are the lipopeptide families of surfactin, fengycin/plipastatin and possibly members of the iturin family. Figure 2 shows the identification of the gene cluster for surfactin production by the antiSMASH software.

In this work, we have confirmed the production of surfactin by the colorimetric method, based on the specific release of fluorescein from the dye complex CPCl-FL (Heuson et al. 2019), previously described by us, which specifically identifies the lipopeptides surfactin, fengycin and iturin. Other previous work of our group, working with the same strain and using Landy's medium, have confirmed the production of the three lipopeptide families by HPLC and mass-spectrometry analysis (Etchegaray et al. 2017). Therefore, to analyze the production of surfactin, which is the major lipopeptide produced in 24 h of growth in Landy's medium (Etchegaray et al. 2017), we have used the CPCl-FL method, assuming that the major lipopeptide produced during the first 24 h is surfactin (Etchegaray et al. 2017; Heuson et al. 2019). Figure 1S (supplementary material) shows a typical calibration curve used for the quantification of surfactin using the CPCl-FL method (Heuson et al. 2019).

We have used two strategies to evaluate the production of surfactin. Initially the growth in modified Landy's medium to reproduce the conditions of our previous work and check for adsorbed surfactin on the biomass. The second strategy was to study the effects of time of growth and arginine supplementation concomitantly by factorial design experiments. In the latter case, the LB medium was used because it is composed of oligopeptides (Sezonov et al. 2007), allowing us to check the rise in surfactin production that is due to the addition of arginine. Table 3 shows the results of surfactin production in modified Landy's medium. The quantification was carried out during the first 24 h. The total amount of surfactin produced in 24 h was 248 mg (in 0.6 L). The biomass obtained agrees with our previous work, approximately 1 g/L (dry weight). Table 4 presents the quantification of total lipopeptide after 72 h of growth. It also shows the amounts of surfactin recovered by the washes with buffer A. The latter determination, including the recovered surfactin adsorbed to the biomass, is 476 mg, considering the total volume of growth media (0.6 L), also yielding approximately 1 g of cells per liter (dry weight).

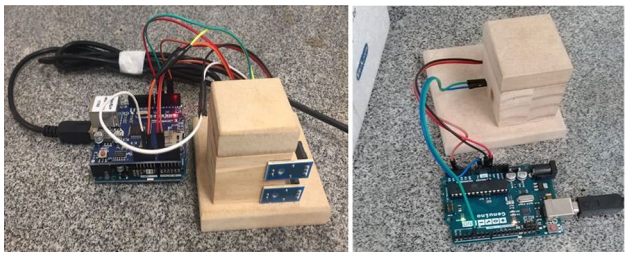

Fig. 1 Pictures of a homemade device used in this work. The device is connected to an Arduino platform. The wood box has a place for the attachment of a cuvette, which has two blue LEDs attached

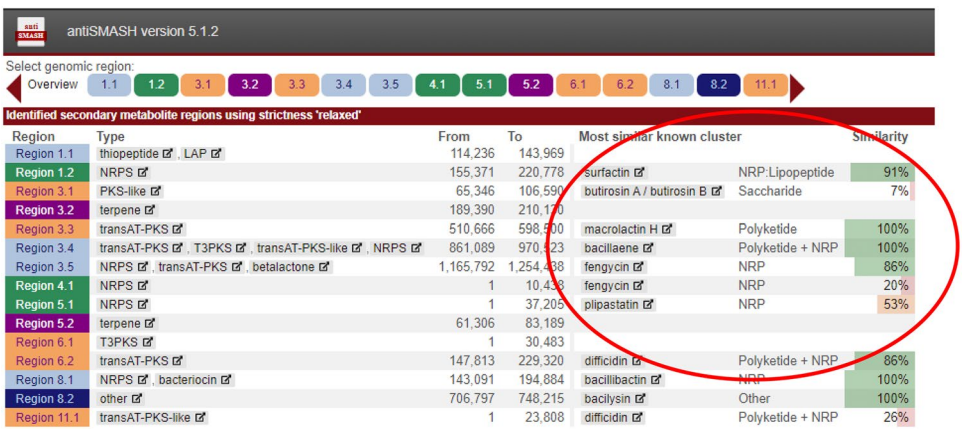

Fig. 2 Results from antiSMASH analysis of the genome of *B. velezensis* strain 0G. The circle highlights the scores obtained for the putative clusters for lipopeptide biosynthesis

Table 3 Yields for surfactin production in modified Landy's medium during the first 24 h

Time	Surfactin (mg/L)				Standard deviation
	Conc 1	Conc 2	Conc 3	average	
12 h	227.7	246.0	236.0	236.5	9.2
18 h	314.3	317.7	317.7	316.5	1.9
24 h	419.3	404.3	414.3	412.7	7.6

Table 4 Total lipopeptide production in modified Landy's medium during the first 72 h

Extract	Surfactin (mg/L)
supernatant	685.4 ± 91.8
Wash 1	55.5 ± 8.9
Wash 2	31.8 ± 2.2
Wash 3	22.0 ± 1.0

Quantified extracts correspond to supernatant and wash-waters, obtained by resuspending the biomass in 50 mM Tris.HCl, pH 7.5

Applications of residues from surfactin production for environmental decontamination of copper

At the end of the growth, surfactin is obtained in the supernatant after collecting the biomass by centrifugation. In this work, we have shown that there is insoluble surfactin adsorbed to biomass. The lipopeptide was recovered by washing the cells with buffer A. Recovered surfactin was added to contaminated samples to remove copper. To evaluate the decontamination of copper, a calibration curve was prepared (Fig. 2S–supplementary material).

The results of decontamination of copper from artificially produced samples, which were treated with biomass and with wash-waters are presented in Table 5. These results demonstrate the application of residues from surfactin production for the removal of copper. Thus, the biomass, containing adsorbed surfactin, and wash-waters, used for surfactin recovery.

In a separate experiment, we have added respectively 40% and 80% more surfactin to a wash-water containing approximately 25 mg/L of surfactin. This resulted in respectively 9% and 12% increase in the capacity to remove copper from solution.

Table 5 Results demonstrating the application of residues from surfactin production to clean up a solution containing copper (Cu^{2+} 320 mg/L)

Residue from surfactin production	Copper (mg/L) final concentration	Copper removal (%)
Wet cell biomass (100 mg/mL)	8.4 ± 0.5	97
Wash-water 1	173.2 ± 8	46
Wash-water 2	157.6 ± 6	51
Wash-water 3	164.9 ± 6	49

Table 6 Decontamination of phenol (hydroquinone 50 ppm) using the biomass of *B. velezensis* 0G

Test solutions	Residue from surfactin production	Average electric current (µA)	Remediation efficiency (%)
Control	None	23.0 ± 1.1	–
Low biomass	Biomass 5% (w/v)	18.0 ± 7	23
High biomass	Biomass 30% (w/v)	11.2 ± 5	51

Applications of microbial cells from surfactin production for the removal of hydroquinone

To explore the application of the microbial biomass, originated from the biotechnological production of surfactins, for the remediation of phenols, a sample of water was artificially contaminated with hydroquinone (50 ppm). This sample was treated with the biomass suspension at low (5%) and high value (30%), weight of wet biomass/volume, which resulted in the removal of approximately 50% of phenol. Table 6 shows the results of phenol remediation by adsorption to microbial biomass. Figure 3S (supplementary material) shows the effect of increasing concentrations of hydroquinone on signal response (current) using the biosensor. A calibration curve was derived from these measurements.

Decontamination of copper monitored by an Arduino controller

To verify the possible application of gravity-induced sedimentation for metal decontamination, we have carried out the sedimentation of copper within a plastic cuvette that is normally used for spectrophotometric analysis. The cuvette was adapted in a homemade device, containing two light sources, positioned at the top and bottom. Absorption was monitored for 24 h through a mobile phone. To transfer the information to the operator and control the

Arduino system, an IOT platform (Blynk) was required. This platform is based on IOS and Android. It generates graphical interfaces allowing the system to send an alert to the operator when the desired values of absorbance are reached Fig. 4S (supplementary material). Figure 5S presents the original copper solution and its mixture with microbial biomass in the plastic cuvette at time zero and after 24 h. The cuvette was left standing within the absorbance chamber at room temperature, and the absorbance readings were recorded over a period of 22 h, as shown in Figs. 3 and 4. At the end of the process, the top layer of the suspension was analyzed by atomic absorption spectrophotometry. The results indicated that the standing time of 22 h induced decontamination by a sedimentation process, allowing the removal of $70.9 \pm 2.8\%$ of copper from solution.

Using the coproduction of surfactin and arginase as strategy to add value to the bioprocess

As in the case of surfactin production, the gene involved in arginase expression was identified after genome sequencing and analysis (described in methods). The open reading frame (ORF) potentially associated with arginase production was named BAC_00197. Sequence alignment of this orf against the database NCBI (Altschul et al. 1990, 1997) returned high

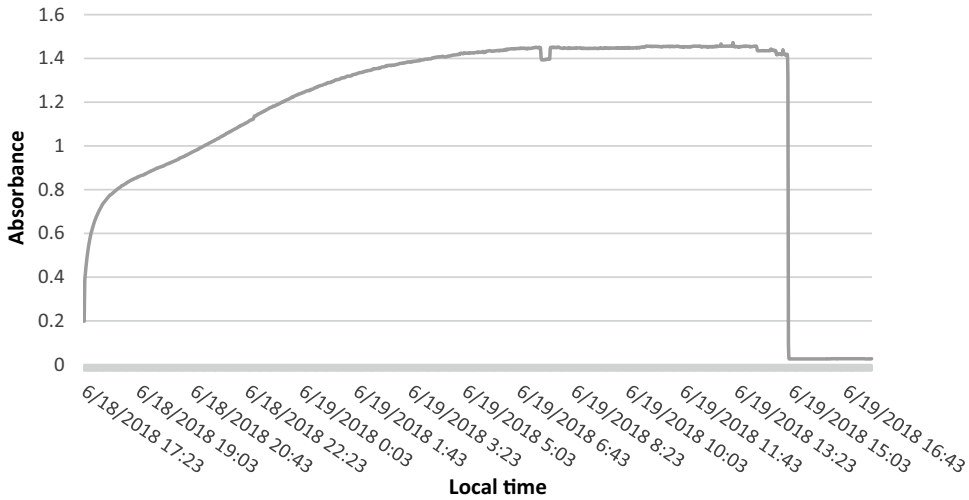

Fig. 3 Absorbance measurements determined during a period of approximately 24 h. Measurements were collected using the top LED of the device presented in Fig. 1

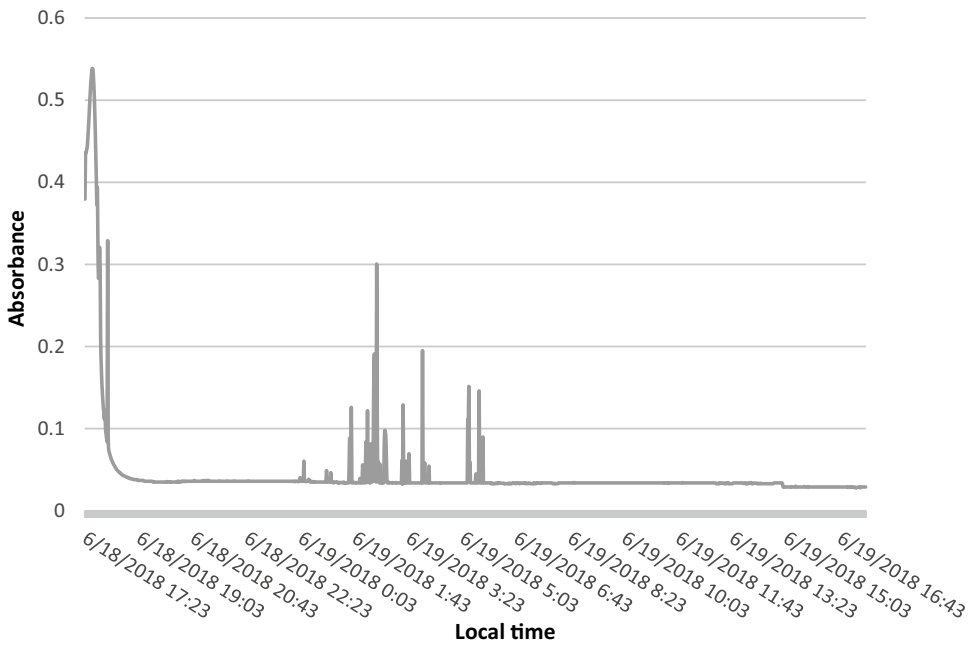

Fig. 4 Absorbance measurements determined during a period of approximately 24 h. Measurements were collected using the bottom LED of the device of Fig. 1

scores for several arginases of other *Bacillus* spp. Thus *B. velezensis* strain OG has the gene that codes for arginase.

Therefore, considering that strain 0G has genes related to surfactin production and a gene coding for arginase, experiments were designed to test a coproduction of arginase and surfactin. According to the factorial design experiments described in methods, the different levels of arginine supplementation in LB medium were prepared, and the growth was carried out for different time intervals. After cultivation, each sample was collected by centrifugation and quantified for surfactin (supernatant), and arginase activity (cell pellet). The results demonstrate that arginine supplementation has positive effects on the expression of arginase and production of surfactin, as shown in Figs. 4 and 5. The surface response curves present a tendency for the maximum expression of arginase and production of surfactin, which are not equal, but are roughly related. Thus, an arginine supplementation around 7 g/L, and growth for up to 24 h.

The response surface curves indicate that the production of surfactin was highest using the parameters: arginine supplementation at 7.27 g/L and cultivation time of 22 h and 29 min. Conversely, the best conditions for arginase expression were achieved at 26 h and 37 min and at an arginine concentration of 6.45 g/L (Fig. 6).

Considering these parameters and the results obtained at the points of maximum production, the expected values for surfactin would be approximately 500 mg/L, considering only the soluble fraction produced during the first 24 h of growth. Similarly, the peak production of arginase was 59 IU, extracted from the collected biomass of 25 mL of growth medium. Therefore, the expected maximum production of arginase at 24 h of growth would be approximately 2,000 IU/L, considering the extraction from the corresponding biomass.

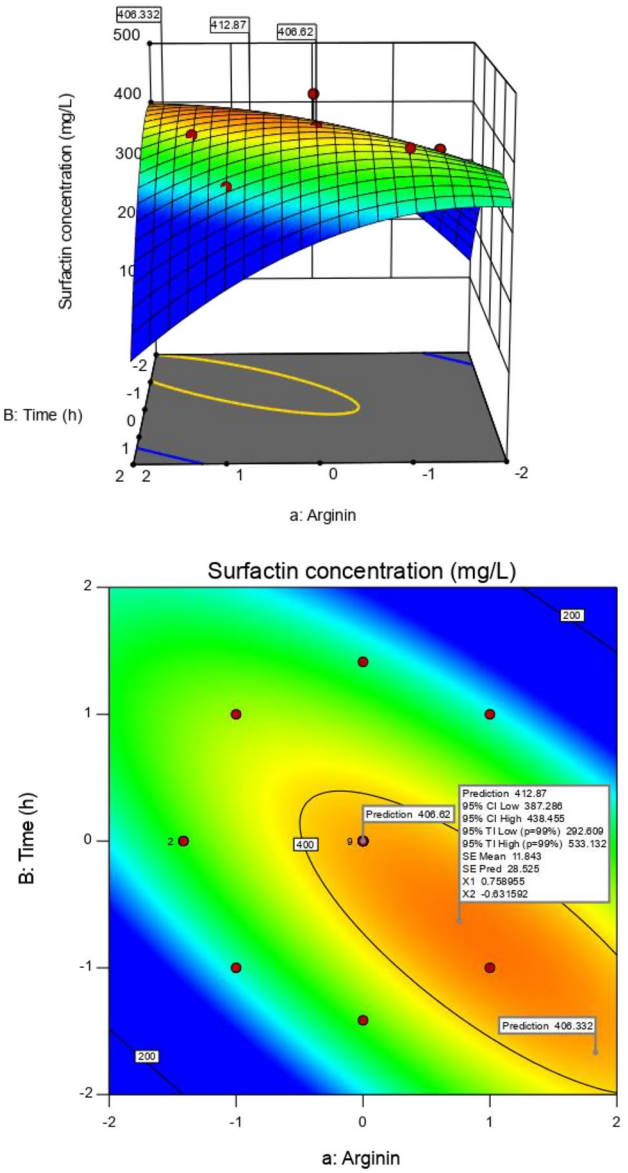

Fig. 5 Response surface curves presenting the best conditions of cultivation to produce surfactin. The curves were produced by the software Design Expert 10.0

Discussion

The biotechnological production of biosurfactants is an expensive process, requiring the search for cheap substrates (Nurfarahin et al. 2018) and the best culture conditions (Etchegaray et al. 2017). Here, we present 3 alternatives that might contribute to lower the production costs associated with surfactin production. Firstly, we have shown that there is a substantial amount of surfactin (~ 16% above the total yield) that adsorbs to the biomass during centrifugation. This result indicates that additional biosurfactant is left attached to residual biomass. We have shown that this can be recovered by extraction with a buffer at pH 7.0. Secondly, we demonstrated that a coproduction strategy can be applied, considering the use of arginine as a source of nitrogen and the fact that arginase may induce the production of glutamate, a substrate used in surfactin biosynthesis (Ginguay et al. 2017). The results indicated, in fact, that supplementation of the LB medium with arginine induced the expression of arginase and production of surfactin. Thirdly, it was demonstrated that microbial biomass, find useful application in copper and hydroquinone remediation. The literature shows that adsorption of contaminants on microbial cells has been used for decontamination (Igiri et al. 2018).

In addition, this work shows that the wash-waters containing surfactin and microbial biomass were successfully used to remove copper from artificially contaminated samples. The wash-waters are more appropriate to remove metals than phenols. Attempts to remove phenol under the same

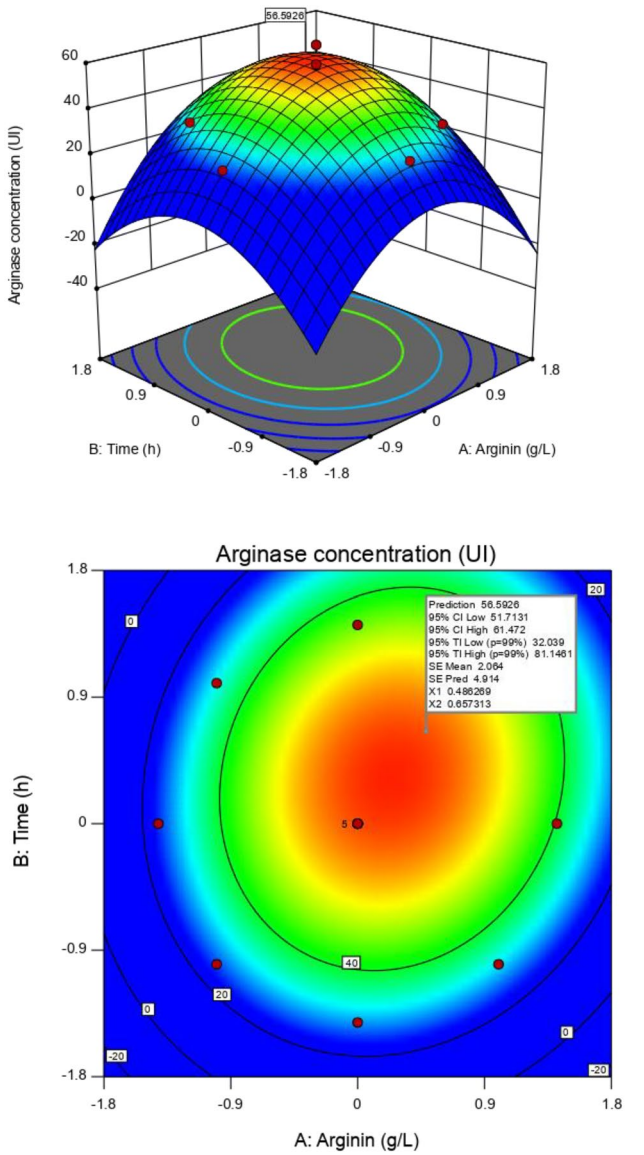

Fig. 6 Response surface curves for the determination of the best conditions of cultivation to express the enzyme arginase. The curves were produced by the software Design Expert 10.0

conditions were unsuccessful, as the micelles that interact with phenol remain soluble. Sedimentation was only achieved at low pH (data not shown). In fact, lowering the pH to reduce surfactin solubility is a normal protocol for its purification (Abdel-Mawgoud et al. 2008). This occurs due to protonation of the carboxylates of aspartic and glutamic acid, both present in the structure of surfactin (De Faria et al. 2011). Similarly, in the presence of metal cations, the double layer of the micelles (Us'yarov 2007) is neutralized by charge interaction with the anionic micelles of surfactin (Açıkel 2011). Under these conditions, the micelles lose solubility. Therefore, the adsorbed metals can be removed by sedimentation. As a result, wash-waters containing recovered surfactin adsorbed to the biomass removed approximately 50% of copper, while microbial cells retained over 90% of copper. Khalid et al. (2015) described a model for the precipitation of metals using anionic surfactants. They suggest that the surfactants could lose solubility by the accumulation of metal cations on the surface of micelles (Khalid et al. 2015). According to this model, the presence of negative charges on the structure of surfactins, is certainly a site of interaction with metals. In fact surfactin naturally interacts with calcium ions, forming salt bridges (Gang et al. 2015). Given that the interaction of surfactin with metals is based on salt bridges, it reduces solubility of the micelles, as in the work of Khalid et al. (2015). These results allowed us to propose a thoroughly sustainable method, from fermentation to the harvesting stage, including coproduction (surfactin and arginase), surfactin recovery from biomass, and environmental decontamination, using the remaining biomass. The precipitation was successfully monitored using an IOT platform. As in the case of other more complex sensor devices, the use of an Arduino platform allowed real time monitoring (Velasco et al. 2016; Antolín et al. 2017; Rajalakshmi and Vidhya 2019).

Besides the application of biomass for the removal of copper and hydroquinone, we also evaluated the production of arginase, an important enzyme that can be used for cancer treatment (Qiu et al. 2015). Arginase is also important in the production of ornithine. Gotoh et al. (2010) identified a process for the direct production of ornithine from casein. It was highlighted the importance of such process, considering the applications of ornithine as a dietary supplement (Gotoh et al. 2010).

Considering lipopeptide biosynthesis, arginase is indirectly involved in surfactin production, as it uses arginine to produce ornithine and urea (Wang et al. 2015). In *B. subtilis*, glutamic acid is a central amino acid, which may derive from arginine and ornithine (Belitsky and Sonenshein 1998). It is also a substrate for surfactin production and is normally added as a nitrogen source in growth medium as in Landy's medium. Previously, we found that the addition of arginine in Landy's medium induced the production of fengycin and surfactin (Etchegaray et al. 2017). Considering that arginine potentially induces the expression of arginase in *B. brevis* and also contributes to the biosynthesis of gramicidin S (Kanda et al. 1997), here, we have studied the best conditions for arginase and surfactin production, using arginine in the growth medium. The LB medium was selected as base medium for these experiments considering that it has no free amino acid. The broth is composed of protein hydrolysates (Sezonov et al. 2007). Therefore, there would be no interference of glutamic acid in the growth medium. Glutamic acid is considered an important source of nitrogen and a substrate for surfactin biosynthesis (Yao et al. 2012; Motta Dos Santos et al. 2016; Etchegaray et al. 2017). Experimental conditions

were based in factorial design experiments. The results indicated that, for surfactin production, the best concentration for arginine in Landy's medium is approximately 7.3 g/L. This value is slightly above the best parameter for arginase expression, which was shown to be best induced at 6.5 g/L of arginine. The best time of growth was also evaluated, and it is in the range of 22 h for surfactin and 24 h for arginase. Considering these results, the growth time of 22 h and supplementation of arginine at 7.3 g/L represents the best choice, based on the use of the synthetic medium LB, and considering that surfactin is the most important component of this biotechnological process. Even though, in this work, we found that this is not the best growth time and concentration of arginine to produce arginase. A possible explanation for a small difference between the expression of arginase and the production of surfactin is that the production of arginase may also induce the production of fengycin, considering the use of ornithine as a substrate (Etchegaray et al. 2017). Ornithine is the primary product of degradation of arginine by the action of arginase (Ginguay et al. 2017).

Therefore, to reduce the production costs of surfactin, we propose the coproduction of surfactin and arginase, the recovery of surfactin from microbial biomass and the utilization of microbial biomass, for the removal of environmental contaminants.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11274-021-03094-3.

Acknowledgements We would like to thank Mr. Mário Henrique Moreno Mr. Ricardo Gimenez for technical assistance. This research received support from Fapesp (13/20570-6 and 17/1330-0). In addition, the work was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001.–scholarship PNPD (Gomes, W.E.). GCT and LHDC were the recipients of scholarships from CNPq (PIBIT-CNPq). TCS was the recipient of a scholarship for postgraduate students from PUC-Campinas. TGB and BPL also received scholarships from PUC-Campinas (Fapic-Reitoria).

Declarations

Conflict of interest We declare that there is no conflict of interest in this work.

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